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# Targeting Developmental Pathways – the Achilles Heel of Cancer?

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## **Running Title:**

Developmental pathways for cancer treatment

## **Abstract**

Developmental pathways (e.g., Notch, Hippo, Hedgehog, Wnt, and TGF- $\beta$ /BMP/FGF) are networks of genes that act co-ordinatedly to establish the body plan, and disruptions of genes in one pathway can have effects in related pathways and may result in serious dysmorphogenesis or cancer. Interestingly, all developmental pathways are highly conserved cell signalling systems present in almost multicellular organisms. In addition, they have a crucial role in cell proliferation, apoptosis, differentiation, and finally in organ development. Of note, almost all of these pathways promote oncogenesis through synergistic associations with the Hippo signalling pathway, and several lines of evidence have also indicated that these pathways (e.g., Wnt/ $\beta$ -catenin) may play be implicated in checkpoint inhibitor resistance (e.g., CTLA-4, PD-1, PD-L1). Since Notch inhibition in vivo results in partial loss of its stemness features such as self-renewal, chemoresistance, invasive and migratory potential, and tumorigenesis, these highly conserved developmental pathways are regarded to be critical for regulation of self-renewal in both embryonic and adult stem cells and hence are likely to be implicated in the maintenance of cancer stem cells. Many small molecules are currently in preclinical and early clinical development, and only two compounds are approved for treatment of advanced or metastatic basal cell carcinoma (vismodegib, sonidegib). Furthermore, therapeutic targeting of cancer stem cells using drugs that disrupt activated developmental pathways may also represent an attractive strategy that is potentially relevant to many types of malignancy, notably blood cancers where the evidence for leukaemia stem cells is well established. Future work will hopefully pave the way for the development of new strategies to target these pervasive oncogenic pathways.

## **Key Words:**

Developmental pathways – novel targets – cancer treatment

## Introduction

Developmental pathways are networks of genes that act co-ordinatedly to establish the body plan. In addition, disruptions of genes in one pathway can have effects in related pathways and may result in serious dysmorphogenesis or cancer. The sum of these genetic events will determine the final outcome. Deleterious genetic events that occur in critical pathways will result in profound abnormalities. The key to establishing which genes are at risk from which exposures lies in understanding the underlying molecular biology of these critical pathways [1,2].

Information of this sort is now becoming available with remarkable resolution and several operating principles seem to be emerging. (i) Pathways, not just genes, are conserved from the model systems (e.g., *Drosophila*) to analogous systems (mouse and rat) and humans. (ii) The pathways make use of several levels of regulation: transcriptional, translational, and post-translational. (iii) Relatively few genes are involved in a combinatorial manner to form complex pathways. And finally, (iv) transcription factors are robustly used to create a pattern for the developing of the body plan [1].

Several developmental pathways have been identified during the last two decades. Amongst them the following pathways have been extensively studied:

- Notch pathway
- Hedgehog pathway
- Wnt pathway
- Hippo pathway
- Others (e.g., bone morphogenetic proteins [BMPs], TGF- $\beta$ , FGFs)

Interestingly, all developmental pathways are highly conserved cell signalling systems present in almost multicellular organisms (plants  $\rightarrow$  tetrapods) [2]. These cascades have been found to play a major role in embryonic development and in tumour promotion. In addition, they have a crucial role in cell proliferation, apoptosis, differentiation, and finally in organ development. Of note, almost all of these pathways promote oncogenesis through synergistic associations with the Hippo signalling pathway [1].

## Notch pathway

The Notch signalling pathway (ligand: delta) is important for cell-cell communications and was found to mediate short range juxtacrine communications [3]. In addition, Notch signalling regulates embryonic development and has a major role in the induction of the mesoderm and cell fate determination (e.g., endocrine, cardiovascular, CNS, and lung development). Despite its multiple roles and versatility, the Notch pathway is relatively simple and conserved across species. In mammals four Notch receptors have been identified (NOTCH 1-4) and five DSL ligands (JAG1-2, delta-like-1, -3, -4). Both receptors and ligands are single transmembrane proteins and will trigger the signalling cascade if cell-cell contact is required [3].

Notch activity is based on the stability and turnover of its intracellular domain (NICD) which is regulated by phosphorylation of the PEST (rich in proline, glutamic acid, serine, and threonine) domain, targeting NICD to proteasome degradation upon recognition by the E3 ligase FBXW7 (F-box/WD repeat-containing protein 7). Interestingly, Notch signalling does not require the use of second messengers [4,5]. The activity is exclusively driven by NICD [6].

NICD phosphorylation will then lead to its proteasomal ubiquitination, turnover, and degradation, defining the half-life of Notch signalling, which then allows the cell once again to become ligand-competent and resetting the signalling for a new cycle of activation [7].

Of note, the domain targeted by NICD is the C-terminal PEST domain which then is phosphorylated by the cyclin C cyclin-dependent kinase-8 complex and glycogen synthase kinase 3 $\beta$  [3,7] suggesting a significant crosstalk between Notch and Wnt signalling pathways in cancer cells

Mutations in the PEST domain, leading to aberrations in NICD stability, are the underlying cause of a number of solid and haematological cancers and different genetic disorders. As consequence, the involvement of Notch signalling in many tumours has led to the investigation of Notch inhibitors (especially  $\gamma$ -secretase inhibitors) as cancer treatments [5] (Figure 1).

## Hedgehog pathway

The Hedgehog signalling pathway regulates morphogenesis of various organs during embryogenesis. In addition, it is also involved in stem cell renewal and organ homeostasis in the adult. Currently, three Hedgehog ligands have been identified: Sonic (Shh), Indian (Ihh), and Desert Hedgehog (Dhh), and the primary receptor for Hedgehog ligands is the membrane protein Patched 1 (Ptch1) [8]. In the absence of a ligand, Ptch1 blocks the pathway activity by inhibiting Smoothend, a transmembrane protein that is a potent pathway inhibitor [8]. Following binding to one of the three Hedgehog ligands to Ptch1, Smo (Smoothend) accumulates in the primcilia and facilitates the activation of GLi (glioma-associated antigne) transcriptional activators and their translocation into the nucleus in order to activate expression of Hedgehog target genes such as GLi1 and Ptch genes [8]. In addition, non-canonical Hedgehog activation has been defined as ligand-dependent Smo activation but independent of GLi activation [9,10]. The non-canonical GLi activation pathways includes TGF- $\beta$ , EGF-R, ras, and PI3K-Akt-mTOR targets [10] which underpins the central role of Hedghog signalling in cancer cells.

Ectopic activation of the Hedgehog signalling pathway is implicated in a wide range of tumours (e.g., medulloblastoma, basal cell carcinoma, etc.). In this regard it is of note that high frequencies of somatic mutations of Ptch1 (70-90%), and to a lesser extent in Smoothend (10-20%) are reported in human basal cell carcinoma [9]. Moreover, some Smoothend inhibitors have been approved (e.g., vismodegib and sonidegib for basal cell carcinoma). In 2012, vismodegib (Erivedge®, Roche, Basel, Switzerland) was the first-in-class FDA- and EMA-approved Smoothend inhibitor for the treatment of locally advanced, unresectable, and metastatic basal cell carcinoma (ORR for advanced tumours: 43%) [11]. In 2015, sonidegib (Odomzo®, Novartis, Basel, Switzerland) was also approved for locally advanced basal cell carcinoma (ORR for 35%) [12]. Acquired resistance, however, is quite common and reactivation of Hedgehog signalling either by mutation of Smoothend or downstream pathway activation is the most frequent form of resistance [8] (Figure 2).

Several literature reports have highlighted that the Hedgehog signalling pathway which regulates developmental processes and organ homeostasis plays a critical role in

carcinogenesis, and studies of cancer stem cells add weight to the proposal that tumours harbour hallmarks of early development in their gene expression repertoire [13]. Since Hedgehog inhibitors block both intrinsic signalling in tumour cells and extrinsic signalling to stromal cells to reduce tumour growth, disruption of developmental signalling in tumours can be of therapeutic benefit. In this regard, targeting the Hedgehog pathway is expected to become a useful tool for treatment of human cancers.

### **Wnt pathway**

The Wnt signalling pathway is highly evolutionarily conserved in animals and is similar across animal species from fruit flies to humans [14,15]. It is crucial for the embryonic development including body axis patterning, cell fate specification, cell proliferation, cell migration, and insulin sensitivity [10,11] and these processes are necessary for the correct formation of important tissues including bones, heart, and muscles [14]. In addition, Wnt signalling was also found to be implicated in carcinogenesis (e.g., breast, colon, etc.) [5].

Without Wnt protein stimulation,  $\beta$ -catenin is anchored by a destruction complex comprised of APC (adenomatous polyposis coli), GSK3 $\beta$  (glycogen synthase kinase 3 beta), and Axin [15].  $\beta$ -Catenin is then phosphorylated by casein kinase 1 $\epsilon$  (CK1 $\epsilon$ ) and GSK3 $\beta$ , followed by ubiquitination by  $\beta$ -TRCP (beta-transducin repeat containing), resulting in proteasomal degradation [14]. Wnt signalling can be suppressed by antagonists such as Wnt inhibitory factor 1 (WIF1), the secreted frizzled-related protein family (sFRP), and Dickkopf (DKK) [16].

Wnt signalling components are commonly mutated in many cancers (e.g.,  $\beta$ -catenin, Axin2, TCF) [17] and oncogenic  $\beta$ -catenin mutations have been identified in malignant melanoma, hepatocellular carcinoma, gastric, pancreas, ovarian, colon, and endometrial cancers [16].

Wnt/ $\beta$ -catenin signalling is also involved in the regulation of tumour immunology. Blockade of programmed cell death (PD)-1 protein and one of its ligands, PD-L1 (also known as B7-H1 and CD274), has demonstrated clinical activity in several types of tumours, mainly

melanoma [18]. Because of their biologic role as regulators of T cell activation, these receptor/ligand pairs have been termed “immune checkpoints”.

Although mainly expressed on (intratumoural) immune cells (especially T cells), tumour cells are often PD-L1 and PD-1 positive and show higher clinical response rates after anti-PD-1, PD-L1 antibody therapies than negatives [18]. However, it seems that the therapeutic benefit of immune checkpoint inhibitors such as anti-CTLA-4 and anti-PD-L1 monoclonal antibodies is dependent on a pre-existing, tumour-specific T cell response in concert with a CD8<sup>+</sup> T cell infiltration [17].

Spranger et al. [17] could show that tumour-intrinsic  $\beta$ -catenin activation led to the exclusion of T cell infiltration into malignant melanoma tumour microenvironment by investigating 266 patients with metastatic malignant melanomas. Further studies using melanoma bearing mice with and without a stabilised, degradation-resistant version of  $\beta$ -catenin suggested that  $\beta$ -catenin activation may exclude the host immune response, e.g. through inactivation of T cells and suppression of dendritic cells (DCs) recruitment, and mediate a lack of T cell infiltration within the tumour microenvironment.

Interestingly, activated  $\beta$ -catenin confers resistance to CTLA-4 and PD-L1 checkpoint inhibitors and appears to be a mechanism being responsible to inhibit T cell-based immunotherapy therapies [17].  $\beta$ -Catenin is also able to block the cross-priming ability in DCs resulting in a decreased CD8<sup>+</sup> T cell response [19]. Since cross-priming is important to produce anti-tumour CD8<sup>+</sup> T cells,  $\beta$ -catenin negatively influences anti-tumour immunity.

Taken together, Wnt/  $\beta$ -catenin signal molecules represent rationale therapeutic targets to overcome resistance to checkpoint inhibitors and restoration of antitumour responses. However, no therapeutic drugs specifically blocking the Wnt signalling pathway are available yet, but important steps have been made in the development thereof. Inhibitors of the Wnt/ $\beta$ -catenin pathway can be classified according to the targets of the agents: Wnt ligands and receptors, the  $\beta$ -catenin destruction complex,  $\beta$ -catenin cytoplasmic–nuclear translocation and  $\beta$ -catenin transcriptional complexes; e.g. Wnt inhibitory factor 1 (WIF1), the secreted frizzled-



related protein family (sFRP), and Dickkopf (DKK) [16,20]. Essential components of Wnt signalling are depicted in Figure 3

### **Hippo pathway**

The core components of the Hippo pathway are conserved from flies to mammals [21]. In humans, these include a kinase cascade initiated by the Hippo kinase MST1/2 associated with the adaptor protein WW45/SAV1, and LATS1/2 in complex with MOB1, which in turn, phosphorylates and inhibits the mammalian transcription co-activator YAP (functions as a co-activator for TEA domain transcription factors [TEAD]) and its related protein TAZ [21]). There are four TEAD family members (TEAD1-4) which have a distinct, but not mutually exclusive expression pattern [22]. YAP plays a critical role in organ size control during development, and its persistent nuclear localization and activation contributes to multiple human malignancies [21].

The Hippo signalling pathway controls organ size in animals and humans through the regulation of cell proliferation and apoptosis and mutations will lead to tissue overgrowth and neoplasia. It is also involved in tissue repair (NF2 as a gatekeeper) and cell-cell interactions. In addition, Hippo and Wnt signalling reciprocally regulate each other's activity through a variety of mechanisms [23]. Moreover, Hippo (via YAP/TEAD) directly regulates Notch components and target genes (active NF2 inhibits E3 ubiquitin ligase CRL4) [24].

Finally, an interaction between Hedgehog and YAP signalling has been demonstrated since Hedgehog inhibitors reduced YAP protein levels [25]. The Hippo signalling pathway is integrated upstream or downstream of other biological processes (e.g., BMP, EGFR, TGF- $\beta$ , Wnt, Notch, and Hedgehog) making it a very interesting target for cancer treatment [26-28].

A dysfunction of the Hippo pathway leads to increased YAP/TAZ activity resulting in oncogenic transformation due to TEAD activation. In addition, many of the pathway components are recognised as tumour suppressor genes, and are mutated in human cancers while YAP/TAZ is classified as an oncogene [27,28]. In fact, YAP/TAZ has been found to be

elevated in some cancers, including breast cancer, colorectal cancers, malignant pleural mesothelioma (70% of all tumours examined), and liver cancer.

Although the mechanisms of driving YAP activation in most cancers are often not clearly understood, downregulation of the YAP protein (e.g., verteporfin) should, therefore, inhibit the assembly of a functional YAP-TEAD transcription factor, suggesting that both, YAP and TEAD might be druggable targets in some cancers with high YAP/TEAD expression (e.g., MPM) (Figure 4). Since in almost all cancers TEAD expression is up-regulated (Table 1) and associated with poor survival, a tumour-promoting role of TEADs has been suggested [28-30].

Amongst all developmental pathways the YAP/TAZ-TEAD signalling pathway was found to be involved in controls organ size in animals and humans through the regulation of cell proliferation and apoptosis by cell-cell contact inhibition [27].

Genetic mutation (loss of NF2 function) leads to a constitutive activation of the YAP/TAZ-TEAD cascade and is found in several cancers including malignant pleural mesothelioma (MPM), hepatocellular carcinoma (HCC), bile cholangiocarcinoma (CCA), gastric and ovarian cancers and other malignancies [26,31].

Given the hypothesis that disruption of NF2 function may be the molecular driver event in MPM, any therapeutic intervention on targets (genes) that are normally kept under control by NF2 (and the Hippo pathway) (e.g., surviving etc.) appears to be a reasonable approach. Mutations in the NF2 gene have been found in approximately 40% of MPM patients [32] and in MPM tumours with no detectable genetic alterations of NF2, its activity is downregulated [23]. Moreover, YAP was found to be constitutively active in more than 70% of primary MPMs and interestingly Hedgehog inhibitors could reduce YAP protein levels which adds weight to the proposal that Hedgehog and YAP signalling are closely linked to each other [23,25].

YAP activation/overexpression is commonly seen in human CCA cell lines and biopsies from patients and is more frequently found in CCA than in HCC, although in more than 85% of CCA patients YAP is mutated [31]. More research is required to identify critical mutations upstream of YAP or indirect YAP regulation by crosstalk with other signalling pathways to provide novel strategies for the therapeutic disruption of the YAP-TEAD axis in CCA.

Zhang et al. [32] have shown that YAP can enhance the malignant phenotype of ovarian carcinoma cell lines and that YAP confers resistance to chemotherapeutic agents commonly used to treat ovarian carcinomas. In addition, they demonstrated for the first time a clear correlation between nuclear YAP overexpression and poor patient prognosis suggesting that the YAP-TEAD pathway may be an attractive therapeutic target [32]. Interestingly, nuclear YAP overexpression was found to be independent of the stage of cancer, thus, ruling out the possibility that nuclear YAP was simply a marker of advanced ovarian carcinoma.

Several lines of evidence that YAP overexpression may be a key marker for the development of ovarian carcinomas have been provided by two other groups of researchers. Steinhardt et al. [33] evaluated YAP expression in the ovary and in ovarian serous adenocarcinoma (most common histologic type). In biopsy specimens from normal ovarian tissue 25% of all patients screened expressed nuclear YAP, whereas 98% of patients with serous adenocarcinomas were found to be positive for YAP [33] ( $p < 0.01$ ). In addition, these authors also provided evidence that nuclear YAP expression between normal lung and lung adenocarcinomas was statistically significant (7% versus 86%, respectively,  $p < 0.01$ ) [33]. Moreover, a significant nuclear YAP expression was also seen in colorectal carcinomas compared with normal colon tissue (79% versus 44%, respectively,  $p < 0.01$ ). In contrast, findings reported for breast cancer did not reach statistical significance [33].

Similar results were found for the cytoplasmic YAP expression ( $p < 0.01$ ). Similar results have been detailed by Hua et al. [34] who demonstrated that overexpression of the Hippo/YAP pathway is involved in the initiation and progression of Fallopian tube epithelial-derived cancer (NB: ovarian serous adenocarcinoma originates from Fallopian tube secretory epithelial cells). In addition, approximately 80% of high-grade serous adenocarcinoma of the ovary were found to have alterations in major components of the Hippo/YAP/FGF loop (down-regulation of LATS1, up-regulation of YAP, TEAD, FGF1,2, FGFRs, and PI3K) which formed the basis for the evaluation of FGFR inhibitors in ovarian cancer in ongoing clinical phase II trials (e.g., Erdafitinib: NCT02699609; BGJ398: NCT02160041; Regorafenib: NCT02736305).

Given the widespread expression of the nuclear YAP in these studies, it is conceivable that the Hippo/YAP pathway is commonly altered in the process of carcinogenesis. Therefore, this pathway could be a potential target for these tumour types that demonstrated higher YAP expression compared to their respective normal tissues.

Currently, no TEAD inhibitor has been approved for cancer treatment, and only very few molecules are in tested in vitro cell models. However, based on the data available so far the YAP-TEAD pathway appears to be a key regulator of cellular proliferation and anti-apoptosis making it an attractive target for future cancer drug development.

## **Others**

TGF- $\beta$  (transforming growth factor  $\beta$ ) acts via specific receptors activating multiple intracellular pathways resulting in phosphorylation of receptor-regulated Smad2/3 proteins that associate with the common mediator, Smad4. Such complexes translocate to the nucleus, bind to DNA and regulate transcription of many genes. The TGF- $\beta$  superfamily of cytokines bind to receptors at the cell surface, and recruits two type I receptors and two type II receptors forming a tetrameric complex. Activated TGF- $\beta$  receptors induce a series of phosphorylation cascades, from receptor phosphorylation to subsequent phosphorylation and activation of downstream signal transducer R-SMADs (receptor-activated SMADs). Phosphorylated R-SMADs form a hetero-oligomeric (often trimeric) complex with SMAD4 (co-Smad). The SMAD complex is then imported into the nucleus and regulates the expression of target genes by direct binding to the target gene promoter and/or through the interaction with transcriptional cofactors in a cell-type-specific manner [35]. Increased expression of TGF- $\beta$  was shown to correlate with a degree of malignancy of many human tumours, and TGF- $\beta$  may also contribute to tumour pathogenesis by direct support of tumour growth, self-renewal of cancer cell initiating stem cells and inhibiting of anti-tumour immunity.

Inhibitors of TGF- $\beta$  signalling reduce viability and invasion of several cancers in animal models and show promises as novel, potential anti-tumour therapeutics [36].

Bone morphogenetic proteins (BMPs) belong to the TGF- $\beta$  family signalling pathway and constitute the largest subdivision of the TGF- $\beta$  family of ligands (encoded by 33 genes and include TGF- $\beta$ , BMPs, and activins) [32,38]. They are unequivocally involved in the regulation of stem cell behaviour as well as in the development and homeostasis of a variety of human organ systems. Moreover, it has been demonstrated that BMPs work in concert with several members of the FGF family (fibroblast growth factor) (e.g., FGF-2) to drive mesendoderm differentiation into liver, pancreatic, cardiac, and haematopoietic lineages [39]. Interestingly, the same study also provided evidence that stem cell differentiation into haematopoietic lineages is highly dependent on the cooperation between BMPs, TGF- $\beta$  and Wnt/ $\beta$ -catenin signalling. To date, the FGF family comprises more than 23 members and many of those have been shown to be crucial for stem cell fate and organ development (e.g., heart) [40].

In addition, TGF- $\beta$  and BMPs together with FGF family members have also been shown to regulate the phenotype and functions of the microenvironment and, therefore, might be useful tools to attenuate pro-tumourigenic microenvironment changes [41] (Figure 5).

Of note, developmental patterning (e.g., limbs) was shown to be controlled by a negative regulation of Hedgehog (Smoothend) by FGF/BMP targets. In addition, the cooperation of BMPs and the downstream transcription factors Sp6 and Sp8 of the Wnt/ $\beta$ -catenin signalling pathway (i.e., via Wnt7A, a key gene for embryogenesis and also being implicated in oncogenesis) has also been demonstrated (extensively reviewed by [42]).

TGF- $\beta$ /SMAD and BMP signalling pathways play important roles in both embryogenesis and oncogenesis. BMP signals are essential for the embryonic patterning and early skeletal formation, whereas TGF- $\beta$  regulates vascular function and angiogenesis (promotion of epithelial to mesenchymal transition) [35]. In carcinogenesis, TGF- $\beta$ /BMP signalling has been shown to have dual functions. It can inhibit cell cycle progression and induce apoptosis and thereby be cytostatic or cytotoxic in tumour cells. However, in advanced cancers in which oncogenic mutations have inactivated these tumour-suppressing functions, TGF- $\beta$ /BMP signalling can induce or enhance invasion and metastasis [36].

The complex functions of the BMP/FDF/TGF- $\beta$  axis in development and disease have also been demonstrated by their dichotomous roles in various tumours and tumour stages [43] suggesting that these molecules may be a valid target for cancer prevention and treatment.

## **Conclusion**

The interruption of the complex web of growth factor signalling is the most important prerequisite in tumour progression. Since of great importance for diverse physiological events during embryogenesis, targeting of developmental pathways needs to be tightly controlled to sustain homeostasis and to avoid detrimental (toxic) responses. Uncontrolled activation of these signalling pathways could lead to unrestrained cellular responses and might result in serious disorders in malignant tumours such as invasion and cancer metastasis. To avoid inappropriate over-activation, multiple negative regulating molecular mechanisms are engaged with mutual feedback and crosstalk circuits that increase the connectivity and complexity of the developmental pathway network. Discovering novel ways of targeting development pathways will, therefore, set the stage for the therapeutic development of specific inhibitors against human cancers. However, when targeting pathways that are as broadly active as the developmental pathways are, the effects on normal tissues have to be carefully monitored. Moreover, it remains to be seen whether the disruption of developmental pathways will be efficacious on the treatment of already established tumours since these cancers might be less dependent on developmental pathway signalling.

Many biological elements including adhesion molecules, cellular metabolic status, TKI-related signalling pathways and developmental pathways (e.g., Notch, Hedgehog, Wnt, TGF- $\beta$ /BMP/FGF) can promote oncogenesis through synergistic association with components of the Hippo signalling pathway ("crosstalk") suggesting that a better understanding of these synergistic effects between Hippo signalling and other biological processes that contribute to carcinogenesis could facilitate the development of new anti-cancer drugs. We might be seeing the start of a new class of anti-cancer drugs – those that interfere with the formation of

complexes that regulate the transcriptional output of a developmental pathway, instead of solely inhibiting upstream enzymatic activities or ligand-receptor interactions.

Finally, it is well recognised that these highly conserved developmental pathways such as Notch, Wnt and Hedgehog are critical for regulation of self-renewal in both embryonic and adult stem cells and hence are likely to be implicated in the maintenance of cancer stem cells [44] and Notch inhibition resulted in partial loss of its stemness features: self-renewal, chemoresistance, invasive and migratory potential, and tumorigenesis in vivo [44].

Therapeutic targeting of cancer stem cells using drugs that disrupt activated developmental pathways represents an attractive strategy that is potentially relevant to many types of malignancy, notably blood cancers where the evidence for leukaemia stem cells is well established. In the case of solid tumours, expression of embryonic stem cell markers such as Oct4 and Sox2 are commonly seen in association with aberrant Notch expression [45] and may promote metastasis by modulating epithelial-to-mesenchymal transition programmes, a process also known to involve Hedgehog signalling. Future work will hopefully pave the way for the development of new strategies to target these pervasive oncogenic pathways.

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## **Conflicts of Interest Statement**

W. Dempke and S. Dale are the employees of Kyowa Kirin Pharmaceutical Development Ltd. (UK). K. Fenchel, P. Uciechowski, and T. Chevassut declare no conflicts of interest.

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## Tables

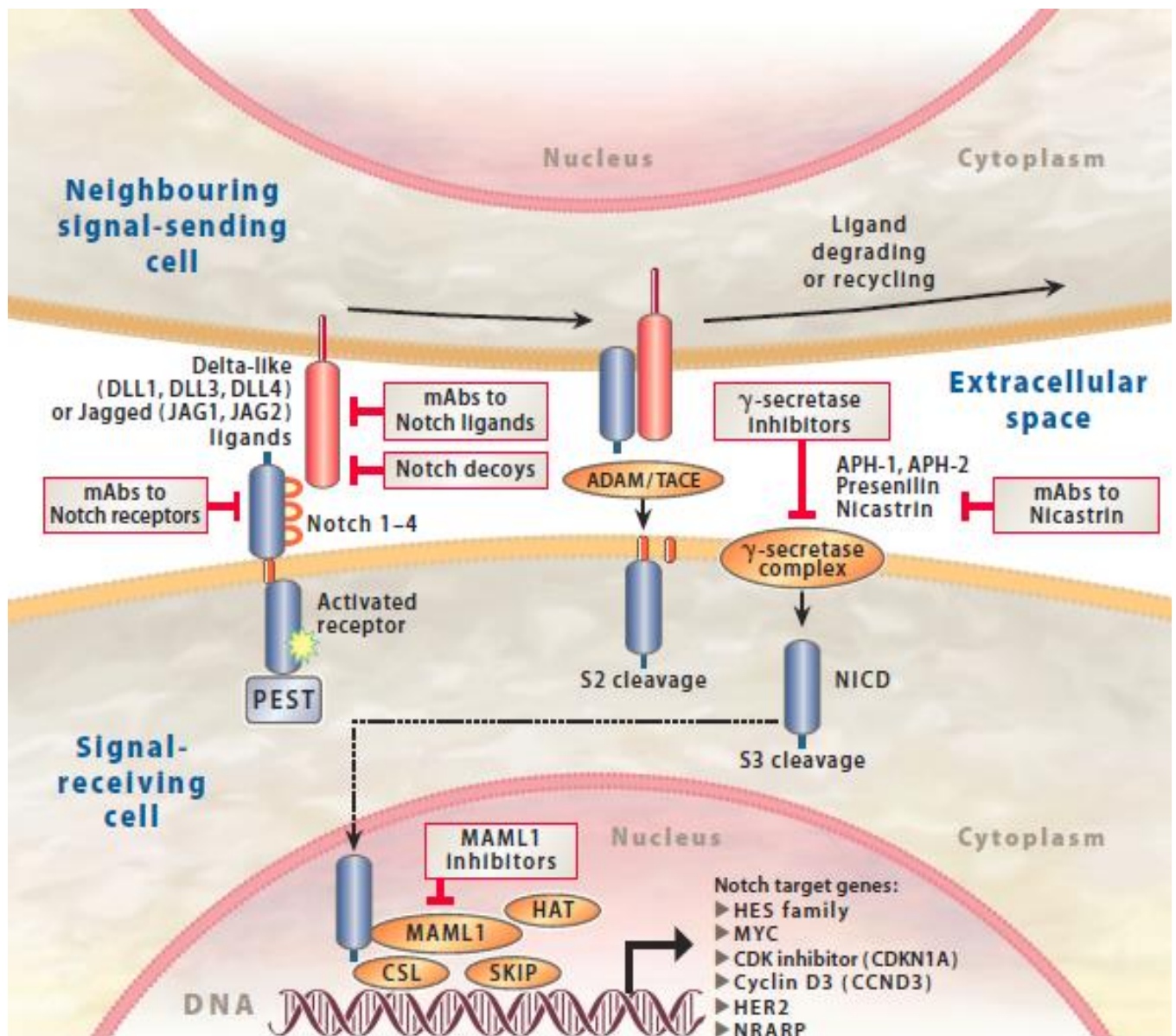
**Table 1:** Summary of the expression of TEAD family members in human tumours. In the vast majority of tumour types a tumour-promoting role of TEADs is suggested. In addition, in some tumours TEAD upregulation correlates with poor overall survival in patients (e.g., gastric, breast, prostate, NSCLC, cholangiocarcinoma, and colorectal cancers [28,32-34]).

Abbreviations: HCC; hepatocellular carcinoma; MPM; malignant pleural mesothelioma; RCC, renal cell carcinoma, NSCLC: non-small cell lung cancer.

<b>Tumour Type</b>	<b>TEAD Family</b>	<b>Gene Expression</b>	<b>Prognostic Marker</b>
<b>Gastric cancer</b>	TEAD-1/4	up-regulated	yes
<b>HCC</b>	YAP-TEAD	up-regulated	yes
<b>Colorectal carcinoma</b>	TEAD-4	up-regulated	yes
<b>MPM</b>	YAP-TEAD	up-regulated	no
<b>NSCLC</b>	YAP-TEAD	up-regulated	yes
<b>Breast cancer</b>	TEAD-4	up-regulated	yes
<b>Fallopian tube carcinoma</b>	TEAD-4	up-regulated	no
<b>Ovarian carcinoma</b>	TEADs	up-regulated	no
<b>Germ cell tumours</b>	TEAD-4	up-regulated	no
<b>Prostate cancer</b>	TEAD-1	up-regulated	yes
<b>RCC</b>	TEAD-1	up-regulated	no
<b>Medulloblastoma</b>	TEAD-1	up-regulated	no
<b>Malignant melanoma</b>	TEAD-1/4	up-regulated	no
<b>Kaposi sarcoma</b>	TEAD-1	up-regulated	no
<b>Bile cholangiocarcinoma</b>	YAP-TEAD	up-regulated	yes

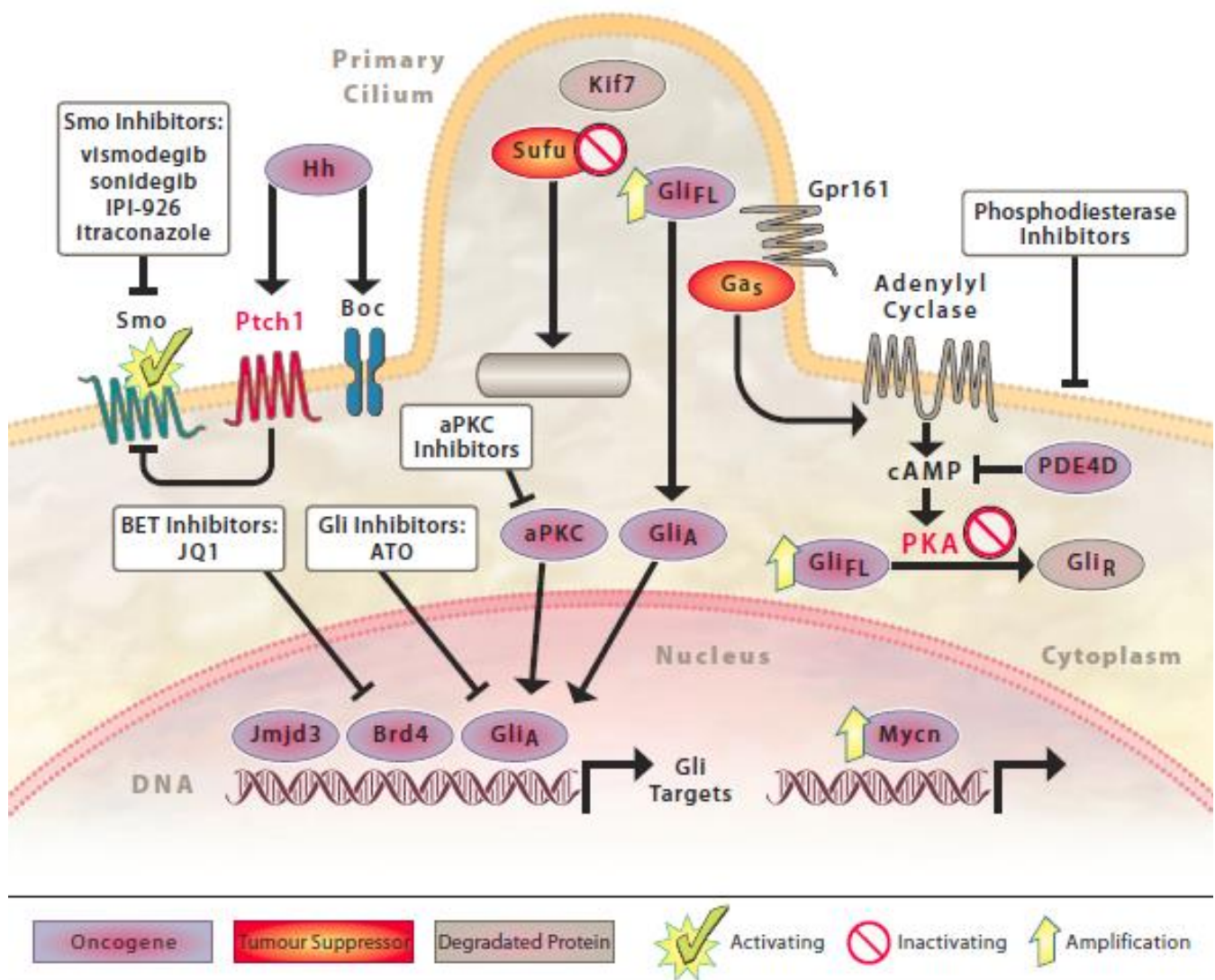
## Figures

Figure 1: The canonical Notch signalling pathway and relevant pharmacological inhibitors under development in cancer. DLL1, DLL3 and DLL4, and Jagged ligands (JAG1 and JAG2) expressed on the cell surface can induce signalling in adjacent cells expressing their cognate receptors Notch1–4. Ligand binding promotes sequential cleavage of the Notch receptors by ADAM/TACE enzymes (S2 cleavage) and then  $\gamma$ -secretase (S3 cleavage), resulting in release of NICD, which interacts with transcriptional regulators in the nucleus to instigate a Notch gene-expression profile. Notch target genes, in turn, regulate pivotal cell-fate choices, including differentiation, cell-cycle progression and survival. The final phenotypic effect is dependent on the specific signalling context, paralogue, ligand and dosage. Under many conditions, and in several types of cancer stem-like cells, Notch signalling can delay differentiation, and maintain proliferative and survival potential. Potential therapeutic inhibitors of targets involved in the Notch signalling include soluble decoy receptors, monoclonal antibodies targeting the Notch ligands or receptors in the extracellular space, and small molecules or monoclonal antibodies inhibitors targeting the  $\gamma$ -secretase complex. Abbreviations: ADAM, a disintegrin and metalloproteinase; APH-1/2, anterior pharynx-defective-1/2; CSL, CBF1/Su(H)/Lag-1; DLL, delta-like ligand; HAT, histone acetyltransferase; HES, hairy and enhancer of split-1; JAG1, Jagged-1; JAG2, Jagged-2; mAb, monoclonal antibody; MAML1, Mastermind-like 1; NICD, Notch intracellular domain; NRARP, Notch-regulated ankyrin-repeated protein; SKIP, ski-interacting protein; TACE, TNF- $\alpha$ -converting enzyme (also known as ADAM17) (modified after [26]).



**Figure 2:** Drivers, drug targets, and resistance mechanisms in oncogenic Hedgehog signalling. Tumour suppressors (red) and oncogenes (green) that have been reported in preclinical and some clinical studies (medulloblastoma, basal cell carcinoma) are shown. Stars (activating or inactivating mutations) and arrows (genomic amplifications) indicate pathway molecules implicated in resistance to Smoothend inhibitors. Hedgehog pathway targeted therapies are indicated in white boxes (modified after [8]).

Abbreviations: Smo, Smoothend; BET, bromodomain and extraterminal domain; PKC, protein kinase C; Gli, glioma-associated oncogene, A: activator; R: repressor, FL: full-length; Kif7, kinesin family member 7; Sufu, suppressor of fused; Ptch1, patched 1; Boc, brother of CDO; Jmjd3, jumonji domain-containing protein 3; Brd4, bromodomain-containing 4; Gpr161; G-protein-coupled receptor 161; Mycn, N-Myc.





**Figure 3:** Components of the Wnt signalling pathway (modified after [5]).

Abbreviations:  $\beta$ ,  $\beta$ -catenin; APC, adenomatous polyposis coli; GSK, glycogen synthase kinase; TCF, T cell factor.

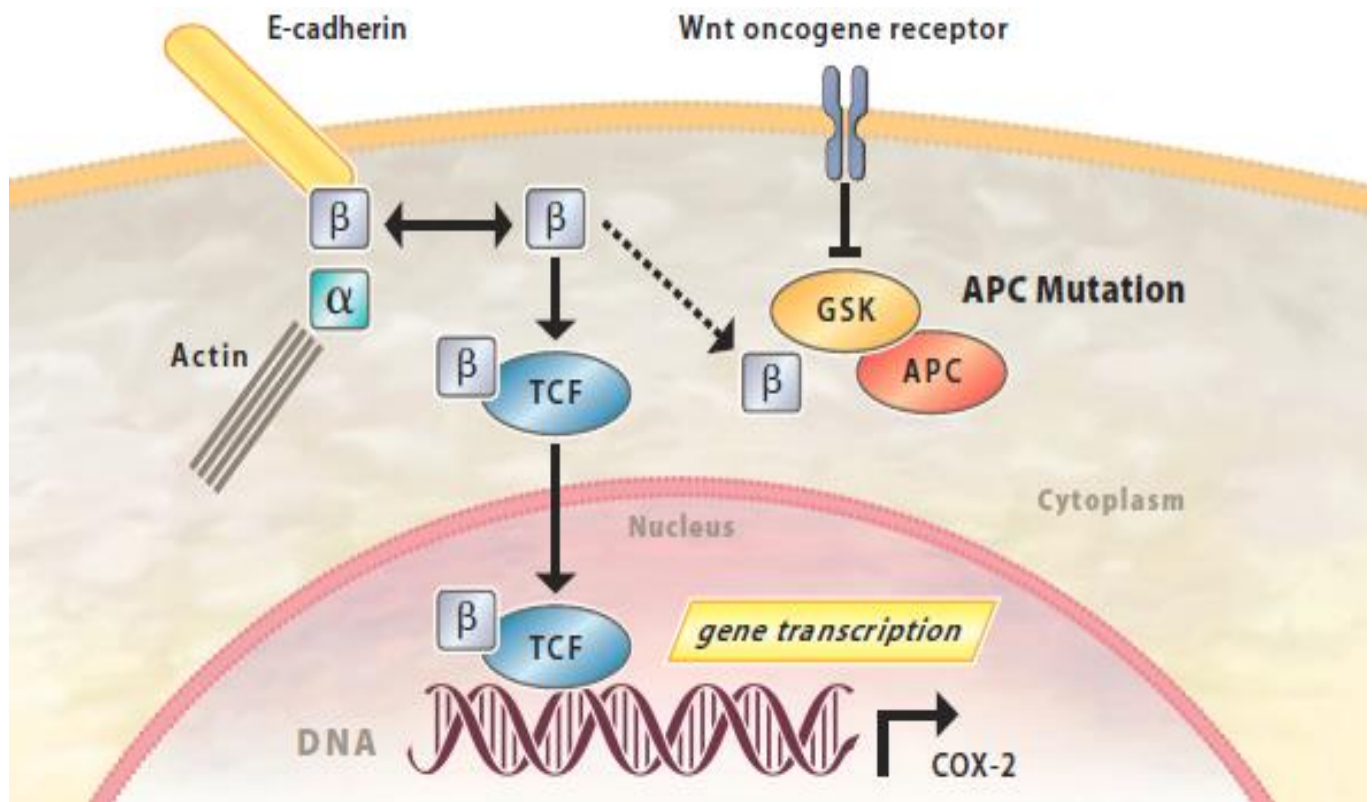
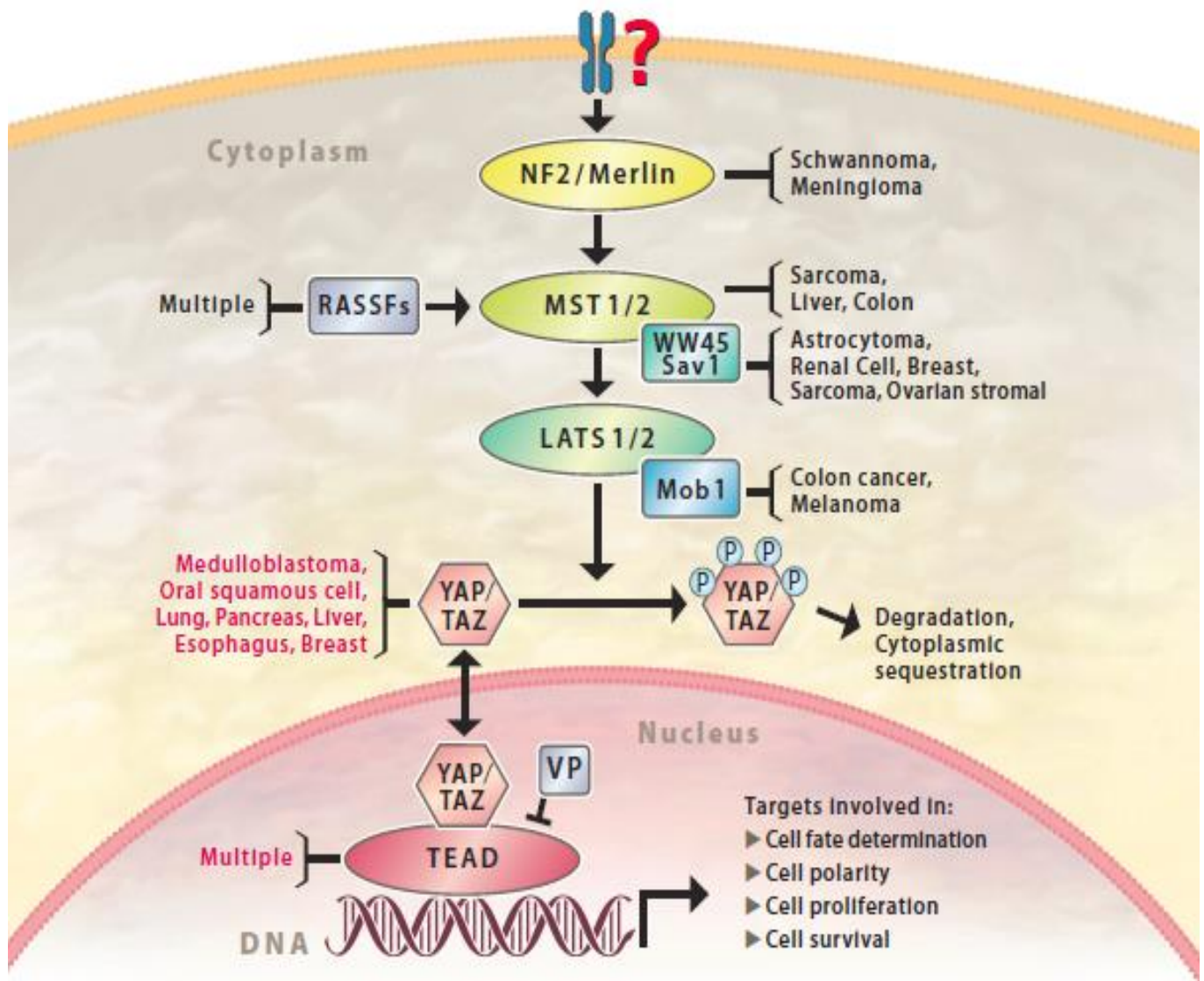


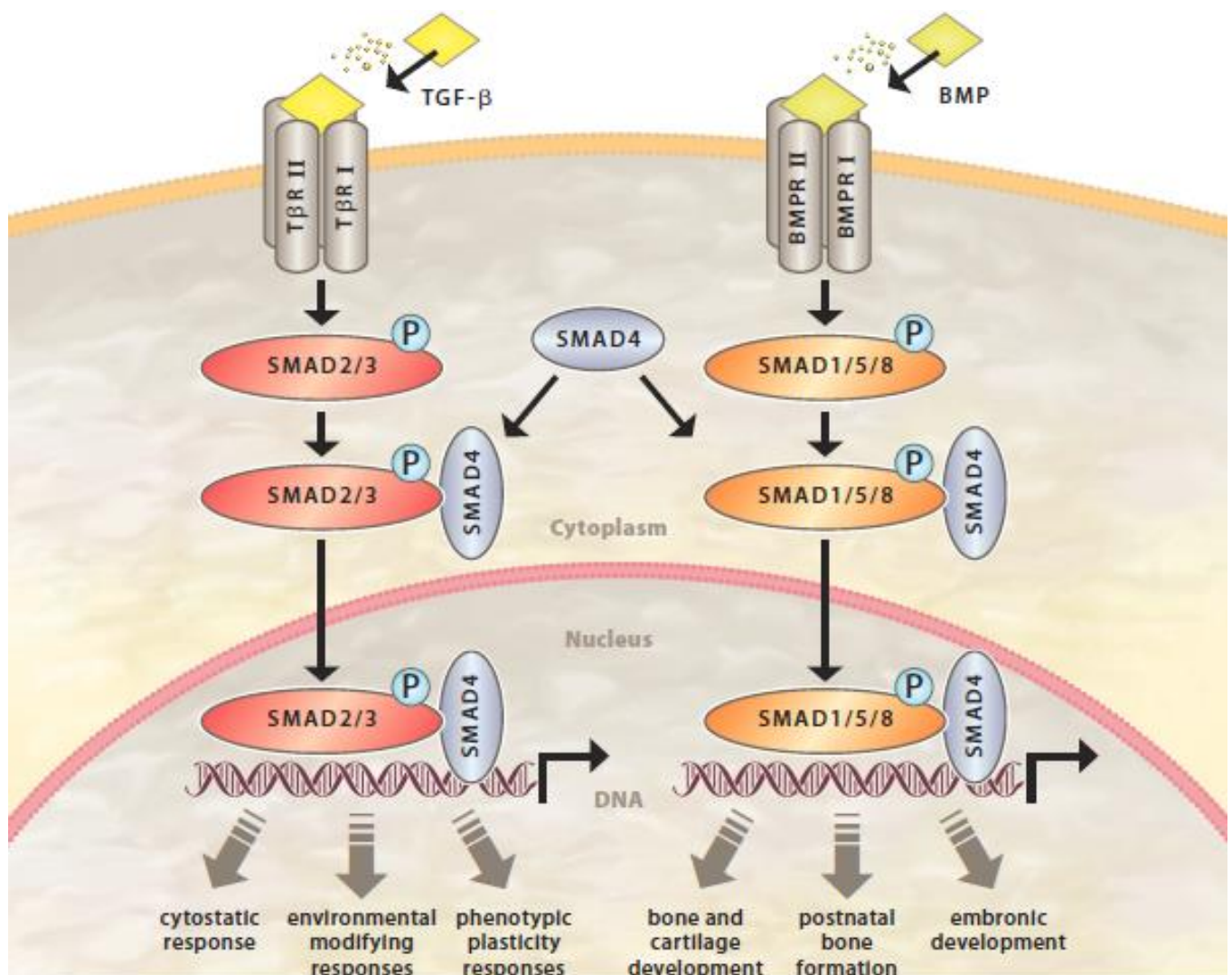
Figure 4: The mammalian Hippo pathway reveals two sets of core kinases, MST1/2 and LATS/2, whose activity is modulated by the NF2/Merlin tumour suppressor, members of RASSF, WW45/Sav1, and Mob1. When the pathway is “active,” phosphorylation of the YAP or TAZ transcriptional coactivators by LATS1/2 results in degradation and/or cytoplasmic sequestration, while pathway “inactivation” allows unphosphorylated YAP/TAZ to enter the nucleus and bind one of four TEAD family members, resulting in context-dependent transcriptional output. In a variety of human (uppercase) and mouse (lowercase) cancers, upstream components of the pathway are down-regulated through frank deletion (NF2) or epigenetic mechanisms (decreased expression shown in blue). YAP, TAZ, and TEAD are up-regulated in a variety of human tumours (increased expression shown in red) by mechanisms that include gene amplification and silencing of upstream components of the pathway. The porphyrin molecule VP disrupts the formation of the YAP–TEAD complex by binding to YAP and changing its conformation, thereby blocking the transcription of downstream targets.

Abbreviations: MST, mammalian Ste20-like kinase); LATS, serine/threonine kinase large tumour suppressor 1; NF2, neurofibromin 2; RASSF, Ras association domain family; WW45, WW domain-containing adaptor 45; Sav1, Salvator 1; Mob1, Mps one binder 1; YAP, Yes-associated protein; TAZ, transcriptional coactivator with PDZ-binding motif; TEAD, transcription enhances activation domain; VP, verteporfin.



**Figure 5:** Signal transduction pathways following TGF- $\beta$ /SMAD and BMP/SMAD activation. TGF- $\beta$  type I, II receptors (also known as activating receptor-like kinase [ALK]) phosphorylate SMAD2 and SMAD3 whereas the corresponding BMP receptors phosphorylate SMAD1, 5 and 8. All these so-called R-SMADs subsequently form complexes with the common partner SMAD4 which then translocate to the nucleus where their target genes are activated or inactivated. Mono-ubiquitination and subsequent de-ubiquitination of SMAD proteins by E3 ubiquitin ligases (e.g., SMURF1,2) play important roles in the control of TGF- $\beta$ /BMP signalling (not shown).

Abbreviations: TGF- $\beta$ , transforming growth factor  $\beta$ ; R, receptor; BMP: bone morphogenetic protein; P, phosphate; SMAD, mothers against decapentaplegic homolog; SMURF, SMAD ubiquitin regulatory factor.





# Fig. 1 "Notch signaling pathway"

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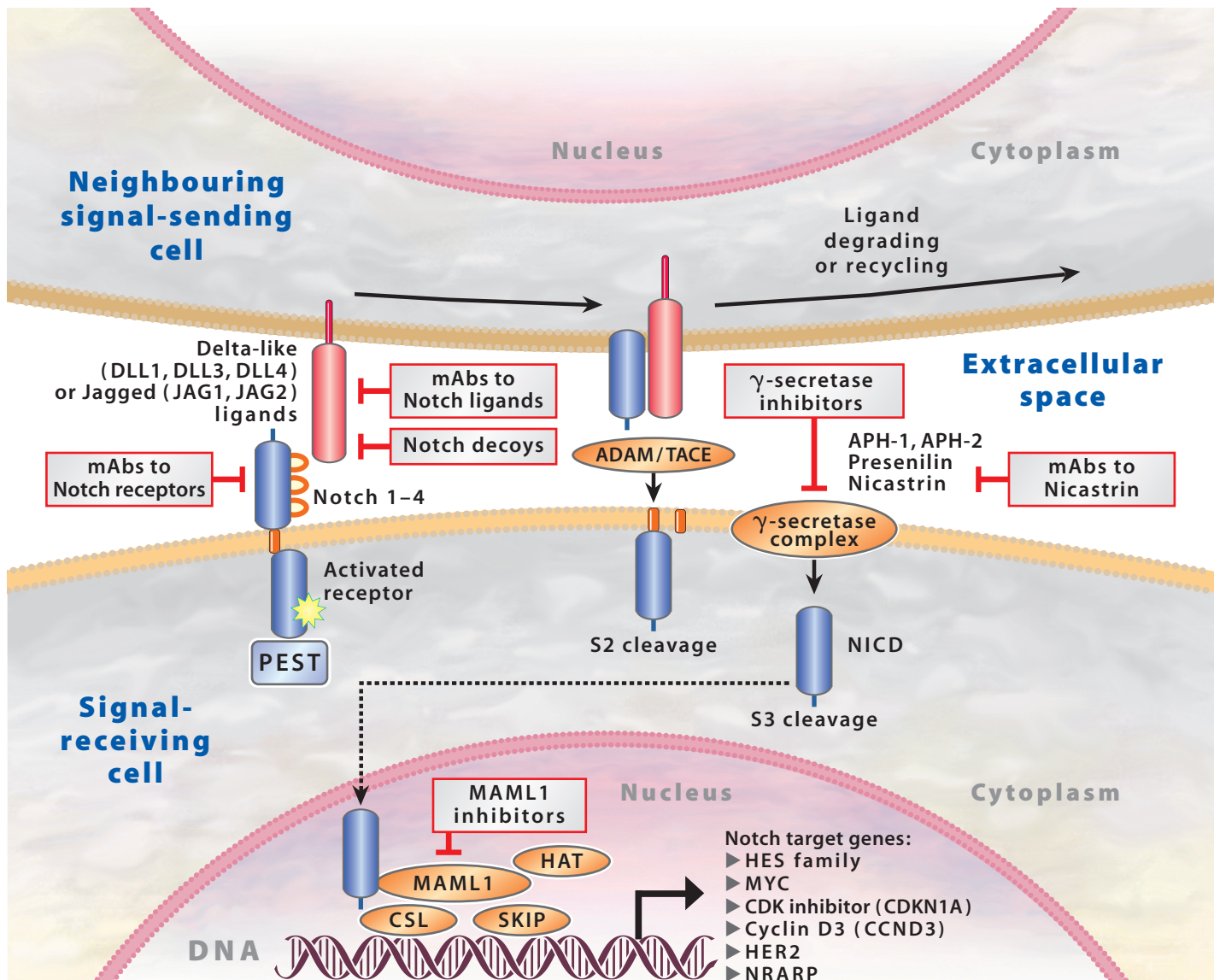


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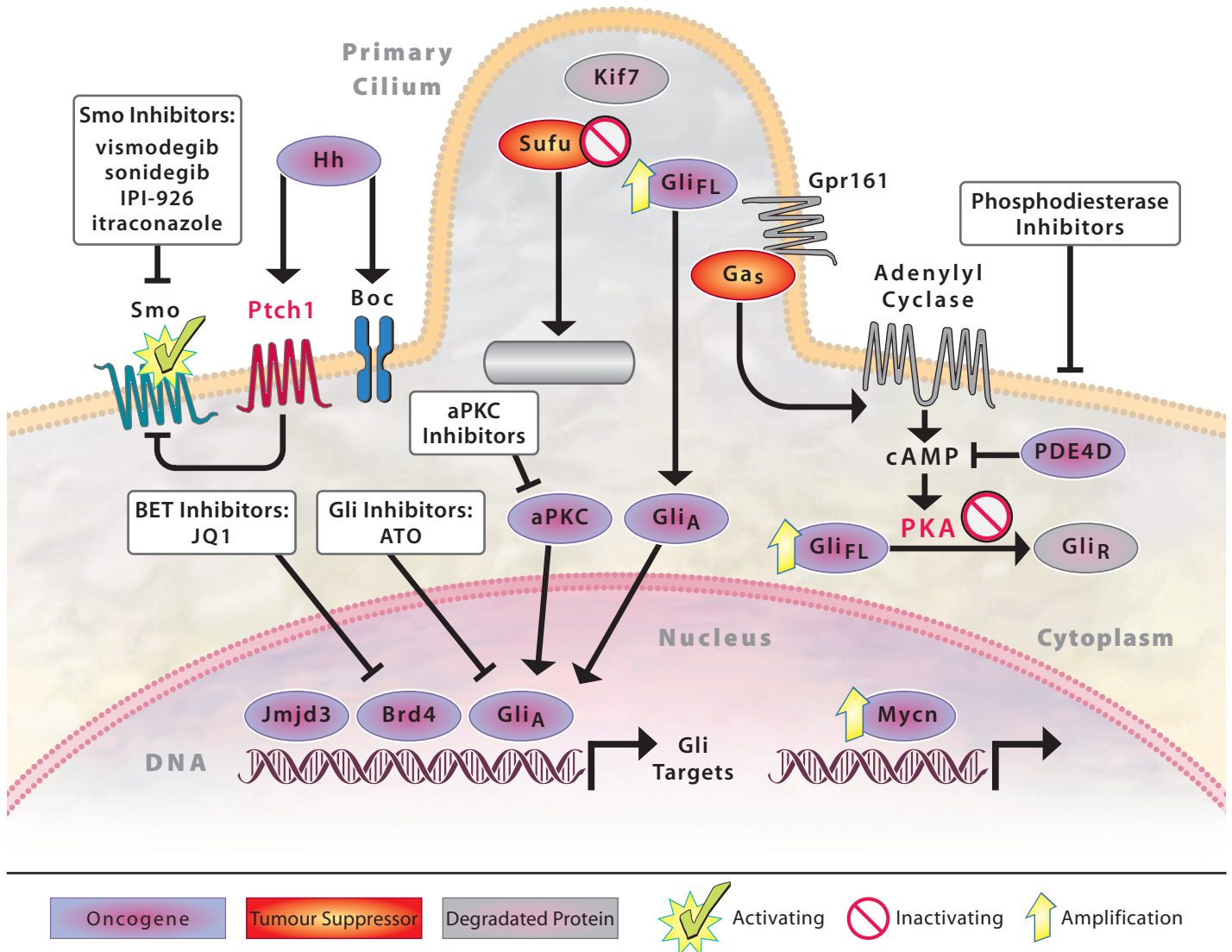
# Fig. 2 "Hedgehog signaling pathway"

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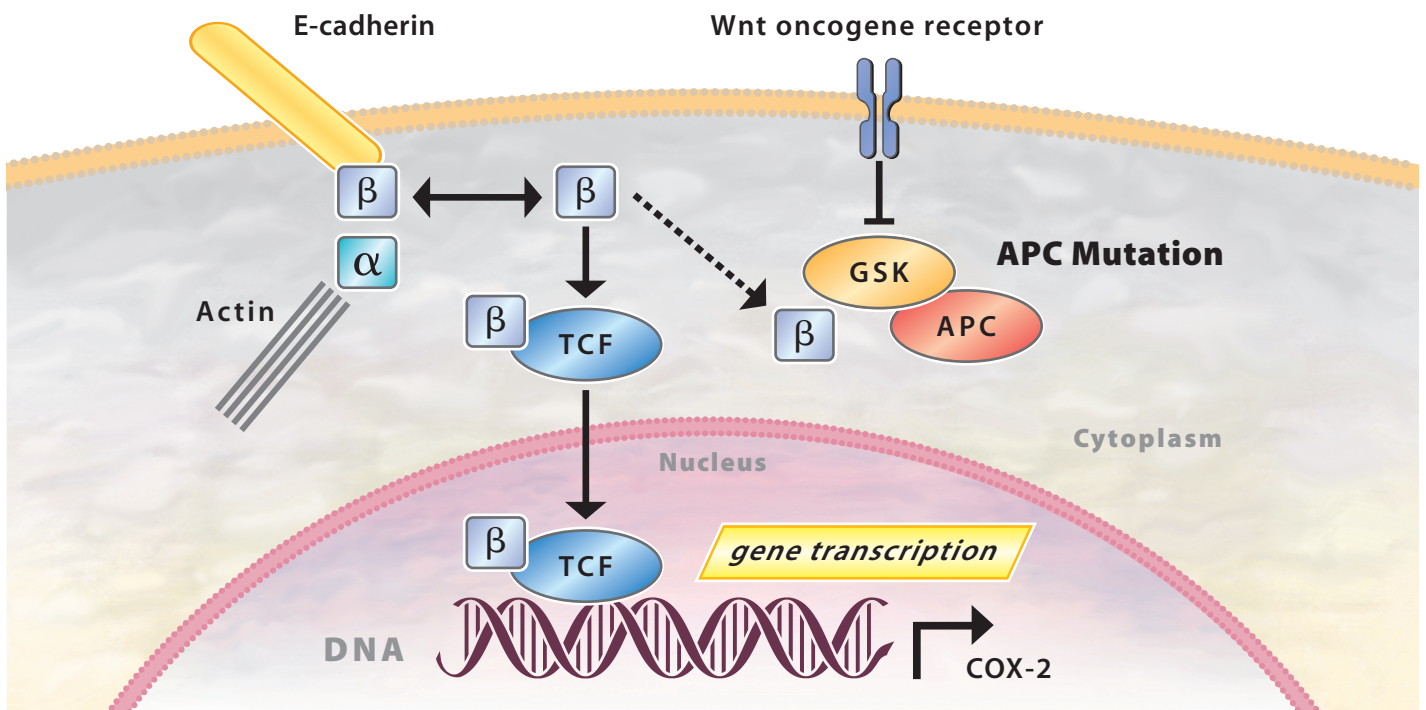


## Fig. 3 "Wnt Signaling Pathways"

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# Fig. 4 "Hippo signaling pathway"

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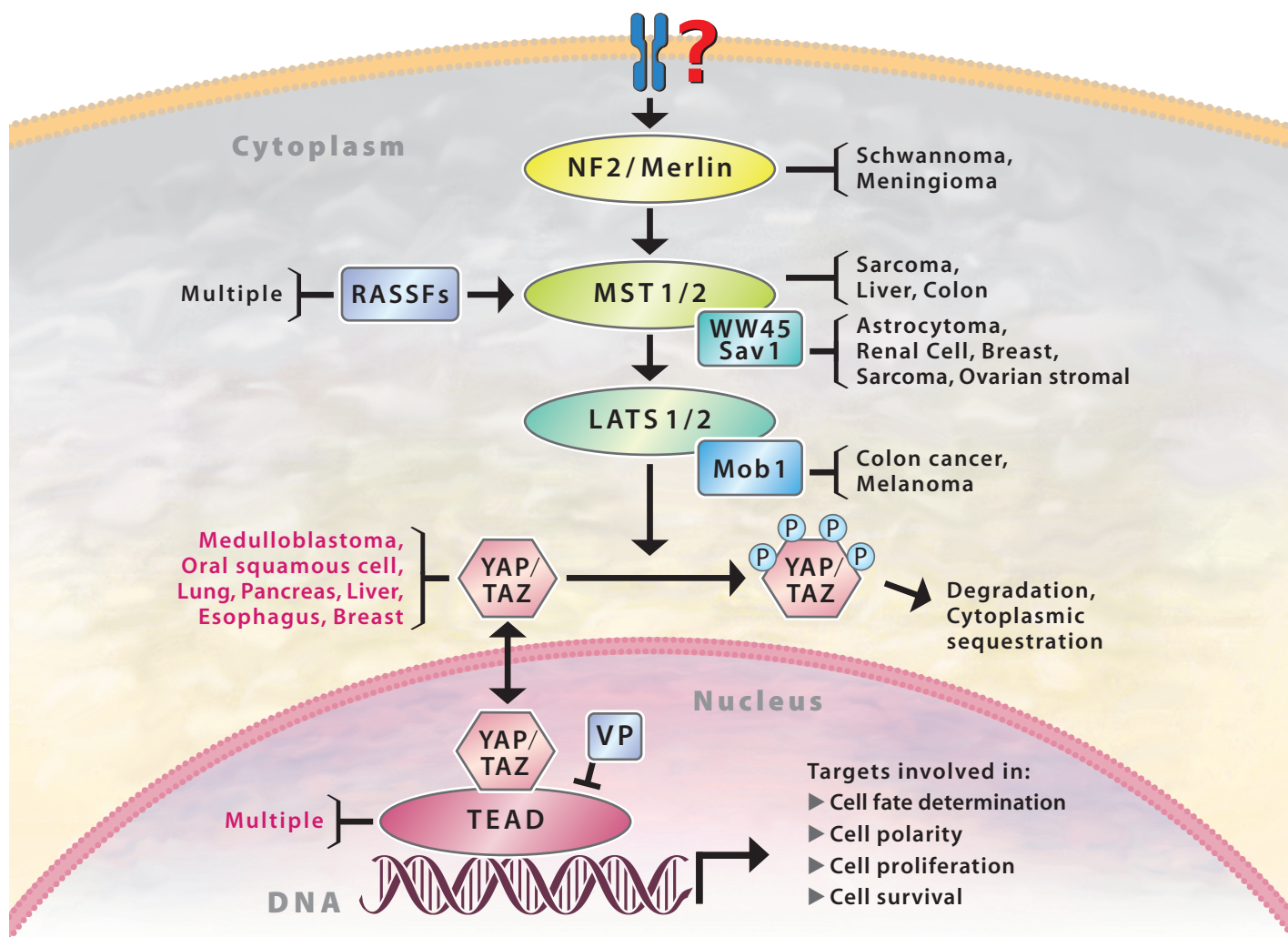




Fig. 5

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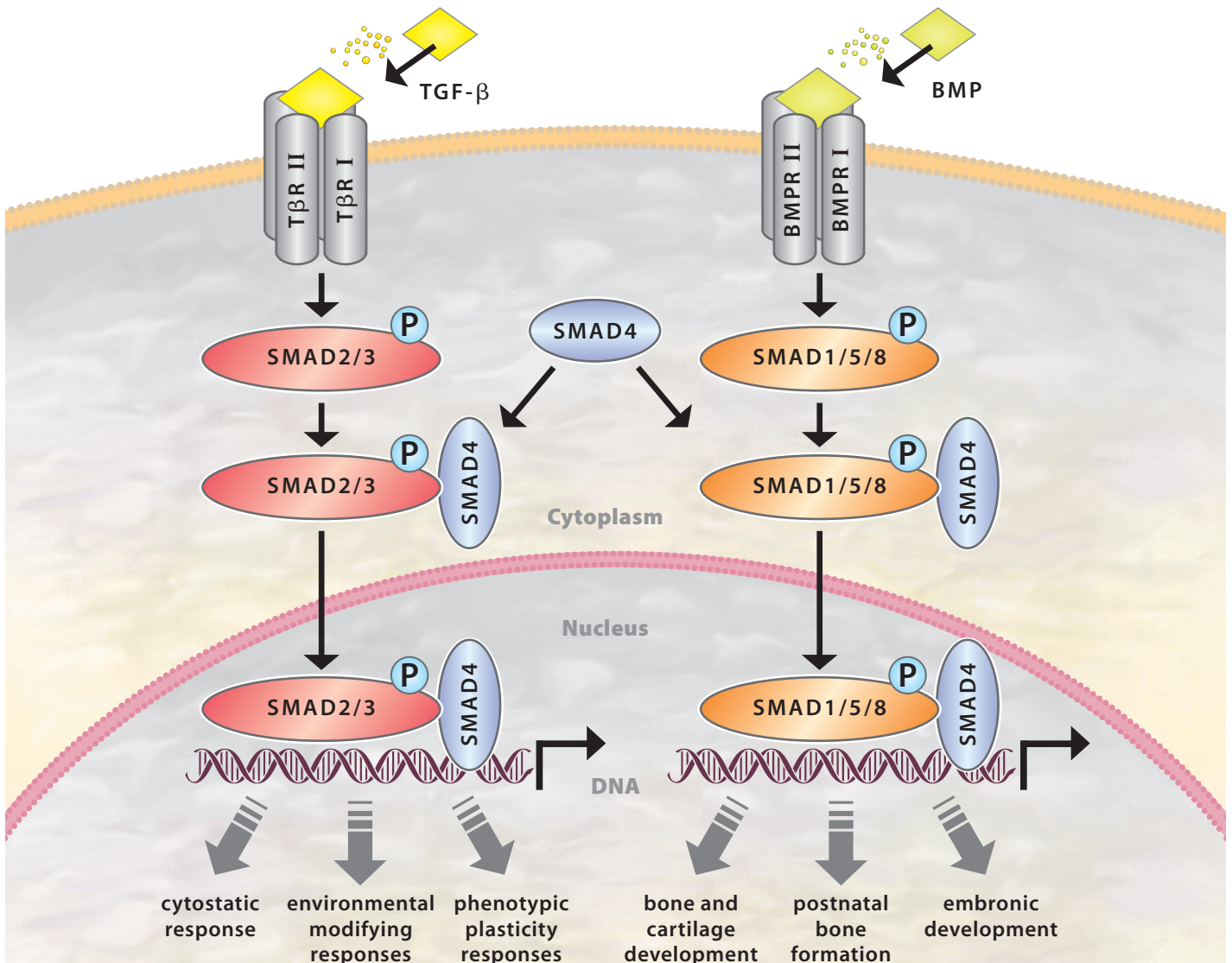


Fig.5 april 2017